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Antioxidant in Oxidative Stress and Neurodegenerative Diseases

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Abstract

Background: *Natural substances have long been used for developing traditional medicines, and the production of innovative drugs is encouraged by the use of these natural ingredients. The key interaction between oxidative stress and inflammation in disease etiology is supported by mounting research. Reactive oxygen species (ROS) generated by inflammatory cells cause oxidative stress, which has been recognized as the key mediator of the relationship between inflammation and the spread for diseases. Curcumin (DFM), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) are the three main components of the rhizomes of turmeric. That filters the superoxide radicals, nitric oxide, and hydrogen peroxide while inhibiting lipid peroxidation, a successful Nano medicine using a curcumin will result in treatment of age-related neurodegenerative diseases such as Alzheimer's and Parkinson's disease.*

Aims: *The goal of this research is to present a new current Nano biotechnological approach to disease treatment and the use of encapsulated Nano cur cumin for treatment of age-related neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.*

Methods: *In the study, amorphous calcium phosphate (ACP) nanoparticles were prepared by the co-precipitation method to optimize release profile of curcumin (Cur) and avoid burst releases, which were used to overcome the weakness of Cur, such as poor chemical stability and bioavailability. To find the best preparation condition, the influence of reaction concentration, temperature, time and pH on crystal phase of the samples was investigated by XRD and FTIR.*

Results: *The results showed that amorphous calcium phosphate (ACP) was obtained, when pH value and the concentration of $PO4^{3-}$ were 8 and 0.024 mM, prepared at 30 °C for 10 min , ACP nanoparticles showed high loading capacity of Cur and favorable pH-responsive drug release properties. Thus the IC50 concentration (µg/ml) of curcumin: Calcium phosphate: Encapsulated NP: Inducer for Alzheimer's disease: and Inducer for Parkinson's disease where estimated respectively.*

Conclusion: *Innovative Nano biotechnology techniques, such as Nano delivery-based strategies, are now being investigated to address current cur cumin bioavailability limitations.*

Keywords: *Oxidative Stress, Neurodegenerative Dseases, Encapsulated Nanoparticles.*

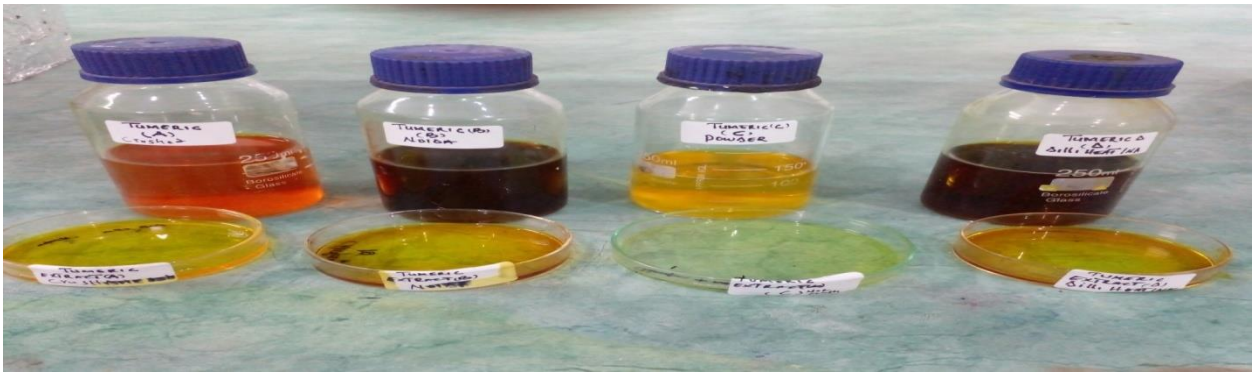
Introduction

Natural substances have long been used for developing traditional medicines, and the production of innovative drugs is encouraged by the use of these natural ingredients. The key interaction between oxidative stress and inflammation in disease etiology is supported by mounting research. Reactive oxygen species (ROS) generated by inflammatory cells cause oxidative stress, which has been recognized as the key mediator of the relationship between inflammation and the spread for diseases.[13,14,18]. Curcumin (DFM), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) are the three main components of the rhizomes of turmeric. That DFM makes up around 77% of the turmeric compound. Although in smaller amounts—17% and 3%, respectively—DMC and BDMC are found in turmeric. Two benzene methoxy rings joined by an unsaturated molecular chain make up the comparable structure that these components share. It originates primarily from India, Indonesia, and other Southeast Asian nations, and it flourishes in tropical places around the globe, as well. It found that from turmeric rhizome, numerous in vitro and in vivo studies have shown the potent antioxidant, anti-inflammatory, antispasmodic, antiangiogenic, and anti-carcinogenic properties of curcumin inhibits the growth factor receptors' ability to function. Additionally Curcumin inhibits cytokines, proteolytic enzymes, eicosanoids, and lipid mediators to reduce inflammation. Curcumin filters the superoxide radicals, nitric oxide, and hydrogen peroxide while inhibiting lipid peroxidation, [2, 5, 10, 12&25]. Thus Curcumin has been used successfully to treat an array of conditions, which includes jaundice and some hepatic diseases, ulcerative colitis, pancreatitis6, irritable bowel syndrome, wound healing, eczema, psoriasis, Alzheimer's, Parkinson's disease, bronchitis, cystic fibrosis, atherosclerosis, hypolipidaemia, and myocardial infarction,[15&20] Additionally, it has potent anti-aging, anti-inflammatory, antiangiogenic, and anti-carcinogenic activities, as demonstrated by numerous human and animal research conducted in vitro and in vivo. Curcumin is regarded as a safe medicine for therapeutic use, and a daily dose of up to 12g is permissible. However, due to a number of factors, including its high hydrophobic solubility and low bioavailability, it has only been used in limited circumstances,[2]. Curcumin's poor solubility in water hinders its pharmaceutical applications, but it works well when digested since the stomach's acidic environment provides curcumin with sufficient solubility,[2]. Nanotechnology has significantly changed the therapeutic viewpoint of many medications, providing fresh and potent options, particularly for the treatment of long-term conditions, numerous cancers, and neurodegenerative diseases, among various others. Nanosystems have the ability to change the pharmacokinetic characteristics of bioactive compounds, particularly those with low bioavailability rates, regardless of their structure and composition, tiny size, and high surface area, [2,7,8, 9&11]. However Drug delivery approaches based on nanoparticles have the potential to make hydrophobic substances, such as curcumin, dispersible in aqueous solutions, hence overcoming the drawbacks of poor solubility, [2, 22&23]. In addition, nano-encapsulated substances showed bioactive capabilities and had cytotoxic and anti-oxidant effects by affecting endogenous and cholinergic anti-oxidant systems. Thus in vitro analysis even at low concentrations compared to free curcumin, nano-encapsulated curcumin has been shown to be an efficient antioxidant in neurodegenerative disorders in neuroblastoma cells line. In the study, amorphous calcium phosphate (ACP) nanoparticles were prepared by the coprecipitation method to optimize release profile of curcumin (Cur) and avoid burst releases, which were used to overcome the weakness of Cur, such as poor chemical stability and bioavailability. To find the best preparation condition, the influence of reaction concentration, temperature, time and pH on crystal phase of the samples was investigated

by XRD and FTIR. The results showed that amorphous calcium phosphate (ACP) was obtained, when pH value and the concentration of PO4 3− were 8 and 0.024 mM, prepared at 30 °C for 10 min. ACP nanoparticles showed high loading capacity of Cur and favorable pH-responsive drug release properties[1,2,3,6,16, 22&23] Thus the IC50 concentration(µg/ml) of curcumin: Calcium phosphate: Encapsulated NP:Inducer for Alzheimer's disease: and Inducer for Parkinson's disease are estimated respectively. The goal of this research is to present a new current Nano biotechnological approach to disease treatment and the use of encapsulated Nano cur cumin for treatment of age-related neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Sample Preparation

Four variety of Turmeric were dried and powdered. 10 gm of turmeric was weighed and Sox let extracted in 500ml of ethanol. 8 cycle for each sample. Extract was evaporated and sample was weighed to calculate. 100 ml of ethanol was added to get a specific concentration.



Fig, 1

Antioxidant assay of extracts

ABTS assay

Ascorbic acid standard: 1mg/ml in water

Ascorbic acid was diluted to 10, 20, 30, 40, 50, 60 µg/ml in water.

ABTS reagent

7mM of ABTS: 0.180 g ABTS in 50 ml of water.

2.45 mM potassium persulphate: 0.033 gm of potassium persulphate in 50 ml of water.

Both the reagent was added in equal quantity and freshly diluted. Incubate for 12 to 16 hrs. to develop colour.

Absorbance of 0.7 was obtained by diluting the ABTS reagent with methanol. Five µl of standards and extracts were added in separate test tube followed by addition of 3.395 ml of diluted ABTs reagent and incubated at room temperature in dark for 4 min. Absorbance was taken at 734 nm. % ABTS inhibition was calculated as:

% ABTS inhibition: ((Ao-At)/Ao)*100

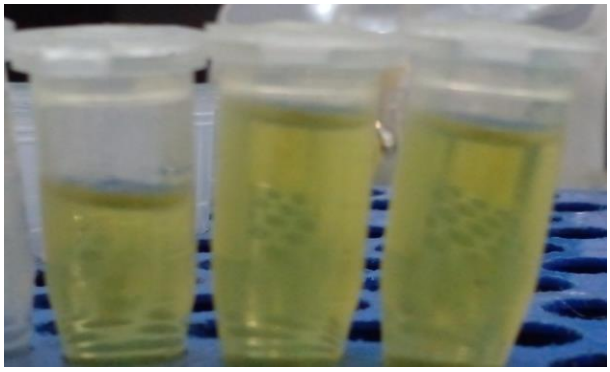
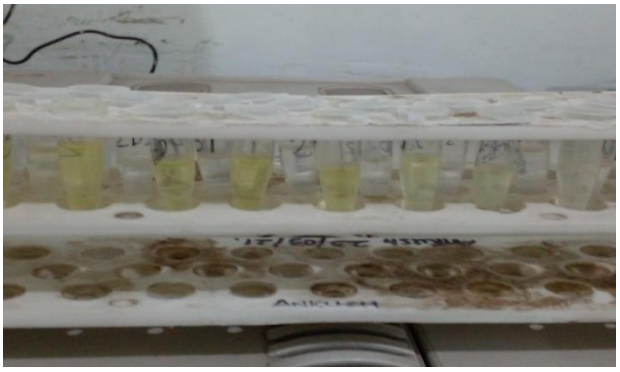
Where Ao: absorbance of control reagent

At: absorbance of test

CEAC (Vitamin C equivalent antioxidant capacity was calculated by linear curve equation from plotting a graph between Absorbance and concentration of ascorbic acid.

Column purification of extracts

Extracts was purified by column chromatography. Briefly silica 60-120 mesh size was added to acetone to make slurry. Column was wet packed. Length of packed column was 20 cm. 5 ml of sample was loaded to top of column and eluted with gradient ratio of acetone and water. Fractions were collected for 5-5 min. Solvent system was: 20 ml of acetone, 16:4 (Acetone: water), 12:8 (Acetone: water), 8:12 (Acetone: water), 4:16(Acetone: water).

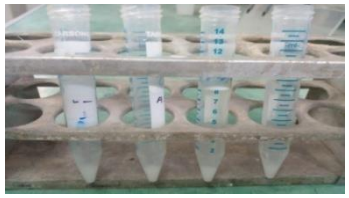
Curcumin concentration of purified fraction in spectrophotometer

Standard curcumin of 1 mg/ml was prepared and diluted in different concentrations. Λmax of curcumin was estimated by scanning the standard curcumin from 400 to 700nm (visible range). The Λmax was found to be 430nm. Absorbance of standards and curcumin fraction was taken at 430 nm. Graph was plotted against absorbance and curcumin concentration. Concentration of curcumin in fractions was estimated by linear curve equations.

Co-Precipitate Method Of Calcium Phosphate Nanoparticle Synthesis

Using the co precipitation method, ACP nanoparticles were synthesized in accordance with (Xia et al. 2018) with a few minor modifications. That Ca (NO3)2 4H2O was turned into an aqueous solution by dissolving it in deionized water. The aqueous solution immediately turned white as the (NH4)2HPO4 phosphate solution was added drop by drop to the previously mentioned solution. With adding NaOH solution, the pH value remained between 8 and 9. The resulting mixture was stirred for 10 minutes at 30 °C. For the purpose to remove any residual ions, the produced nanoparticles were washed three times with deionized water. The sample was then collected by centrifugation and freeze-dried.

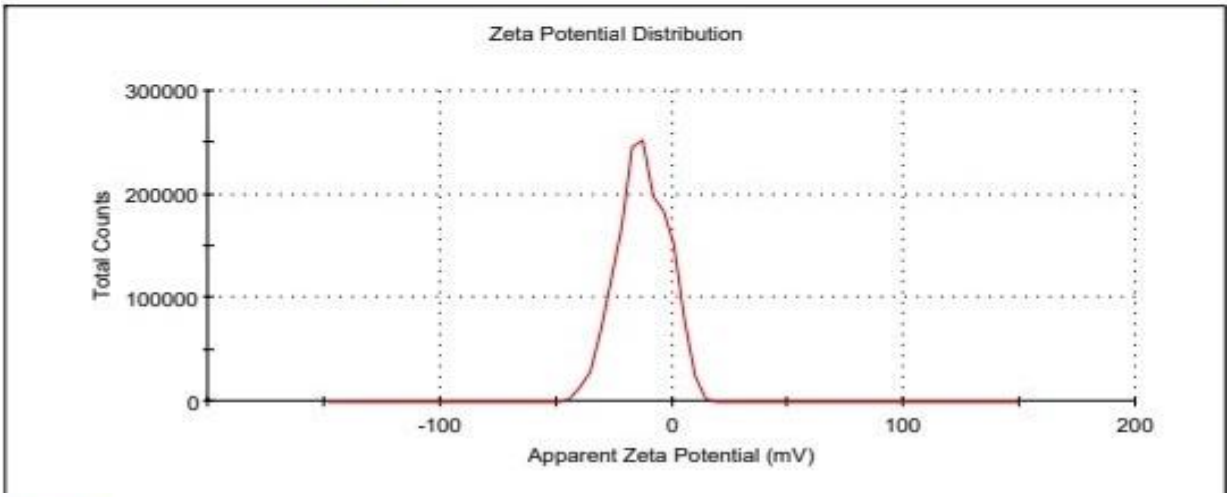
Drug loading: Cur (0.5 mg/mL) was added to the solution of Ca $(NO3)_2$. The mixture was stirred for 1 h, An aqueous solution of Na2HPO4 was then gradually added, and the mixture was gently agitated at 30 °C for an additional 15 minutes. After the suspension was immediately centrifuged to separate the products.

a 
b 
c 

Characterization of calcium phosphate nanoparticles encapsulated with curcumin nanoparticles

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-12.6	Peak 1:	-12.6	100.0	10.8
Zeta Deviation (mV):	10.8	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.268	Peak 3:	0.00	0.0	0.00
Result quality	Good				



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	70.45	Peak 1:	113.2	83.6	40.93
PdI:	0.663	Peak 2:	13.01	11.8	2.838
Intercept:	0.952	Peak 3:	4871	4.7	686.8
Result quality	Good				

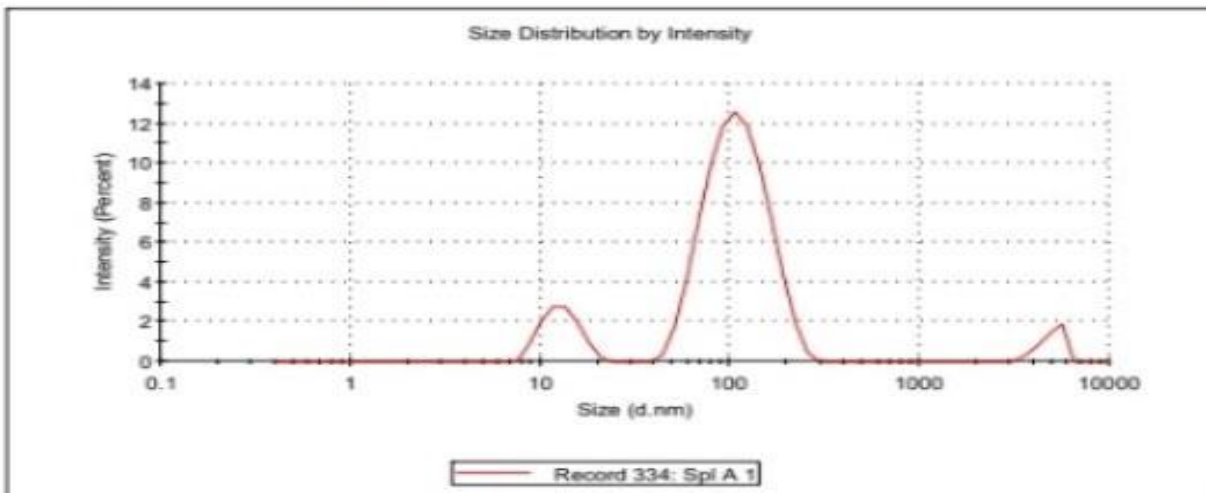


Fig. 4a and b present good quality of the nanoparticles having Pdi of 0.663, Z-average (d.nm) 70.45 as indicated by the DLS machine.

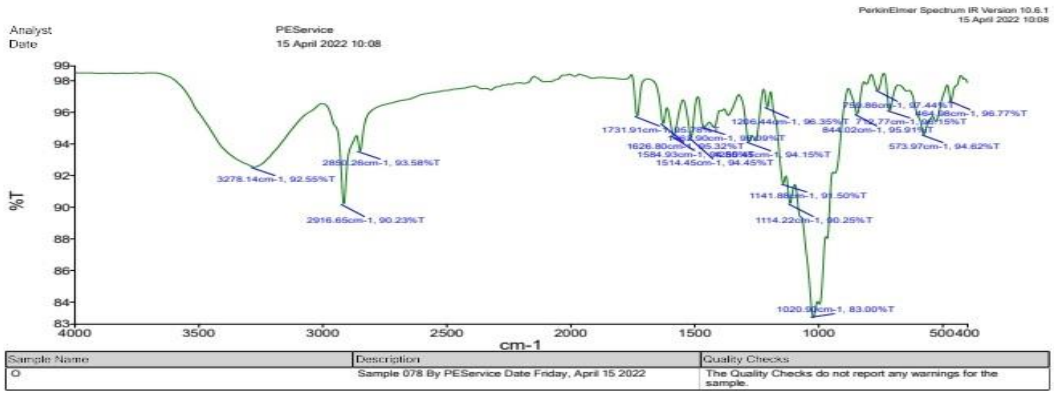


Fig.4c. point out curcumin's infrared spectroscopy. Arrows point to the molecule's characteristic bands.

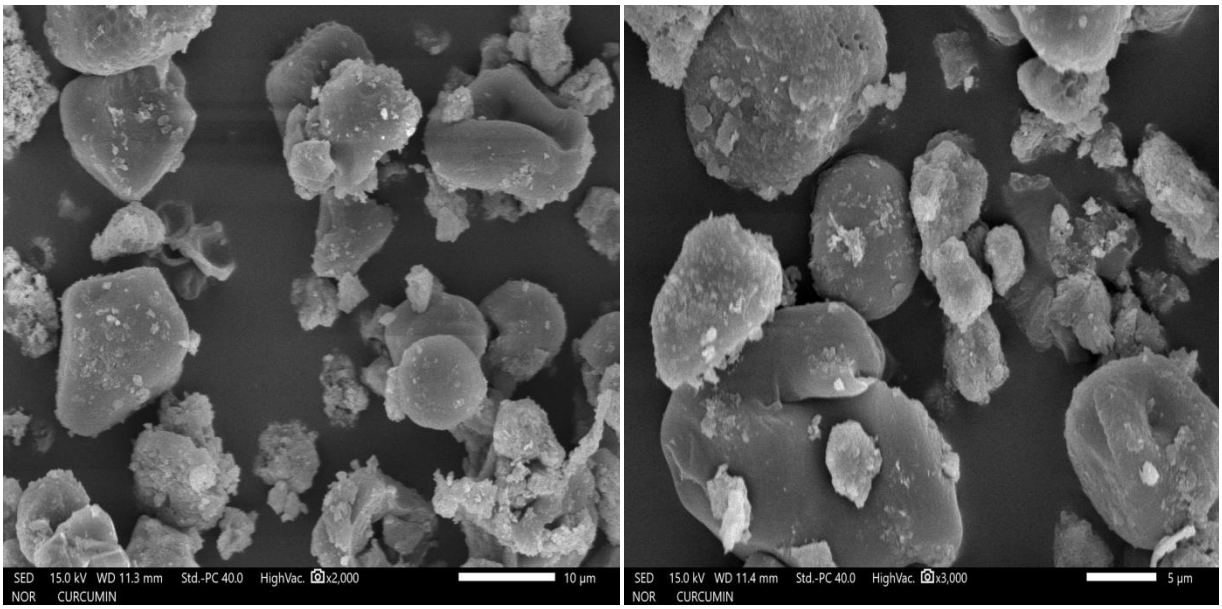


Fig. 4d and e. Demonstrate the encapsulated nano curcumin AMP .

SH-SY 5Y Cell Line

Sh-sy5y neuroblastoma neurodegenerative Cell lines were procured from NCCS, Pune. Cells were sub cultured in DMEM media.

Sub culturing of cell lines

examinations of cultures is accomplished by employing an inverted microscope to check for confluence and bacterial and fungal contamination. Away with the used media. The cell monolayer was washed in PBS devoid of Ca2+/Mg2+. Repeat this wash step if the cells are known to adhere aggressively. Pipette 1 ml of trypsin/EDTA per 25 cm2 of the cleansed cell monolayer's surface area. Rotate the flask to apply trypsin to the monolayer. Remove any extra trypsin. For two to ten minutes, put the flask back in the incubator. Examine the cells under an inverted microscope to make sure they are all floating and unattached. The flasks' edges should be softly touched to remove any remaining adherent cells. Resuspend the cells in a small volume of fresh serum-containing medium to inactivate the trypsin. Take 100–200 l out, then count the cells. Transfer the required number of cells to a fresh, labeled flask filled with warmed medium (the necessary seeding density may be found on the relevant ECACC Cell Line Data Sheet). Following the directions, incubate the cell line. To accommodate the cell line's growth characteristics, repeat this procedure as needed.

The cells were subcultured in 20 ml of DMEM medium with 10% FBS and incubated at 350°F in

a CO2 incubator with 5% CO2 and 95% humidity. After reaching 70–80% confluency, cells were trypsinized and the MTT assay was performed.

Cell Preparation

To avoid losing suspension cells, transfer the media into a centrifuge tube. After incubating the adherent cell for 10-15 minutes, wash it with 1XPBS 2X10 ml. Pour the PBS. Add the necessary amount of Trypsin/EDTA to cover the surface of the T-flask, preferably 1 ml. for 10 to 15 minutes, occasionally tapping. Seen under a microscope to see glued cells completely separate. Place everything in a centrifuge tube. Centrifuge it for 10 minutes at 10,000 rpm. Discard the medium and stir the pellet into 1 or 2 ml of DMEM media that has been warmed. Trypan blue is used to count the cells under a standard microscope.

Count the cells after adding 20 ml of DMEM media.

MTT assay

IC50 estimation for the samples

Using a few low modifications, the MTT colorimetric test method was used to gauge the materials' cytotoxicity. 2*105 cells were used to seed each well before it was incubated for 24 hours at 37 degrees with 5% CO2. The test samples were then added to a confluent monolayer plate containing cell lines that had been grown for 24 hours at concentrations of 160, 120, 80, 40, and 200 g/ml. After 72 hours of incubation at 37°C with 5% CO2, the supernatant was collected, and 25 l of MTT reagent (2 mg/ml) was applied to each well. Each well received 100 l of dimethyl sulphoxide to solubilize the formazan precipitate during a 4-hour incubation period at 37°C. The wells were then stirred for an additional 15 minutes. The absorbance at a 490 nm wavelength was calculated using an ELISA reader. The control wells received the medium minus the test substance. The concentration of the tested sample required to inhibit 50% of the growth of the cells was calculated to reflect the percent inhibitory activity of the tested sample as the IC50 value of cellular growth inhibition.

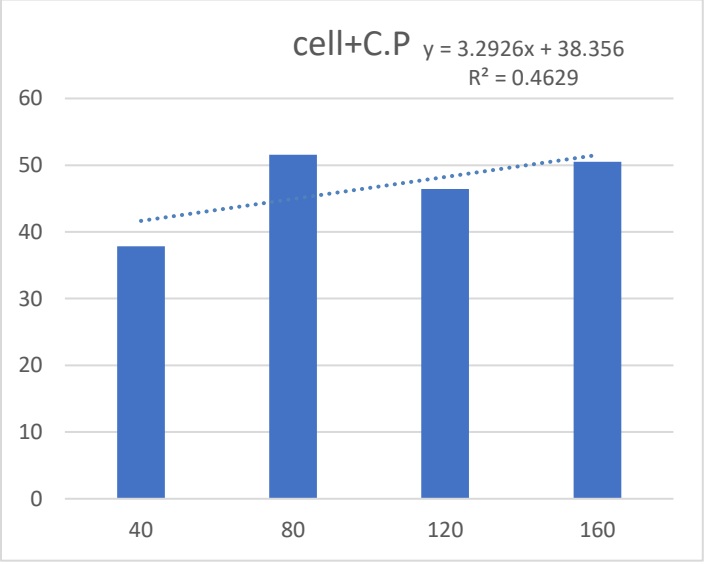
(100*[A0-A1])% inhibition.

where;

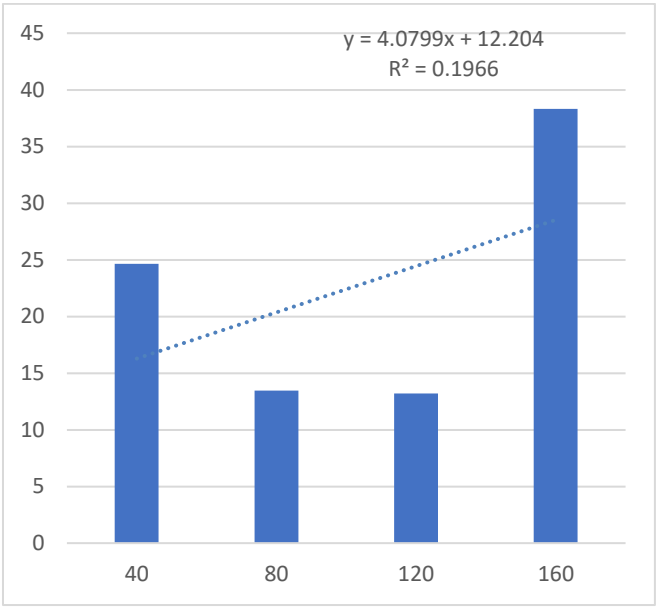
A0 is the absorbance of the control and

A1 is the absorbance of the extracts.

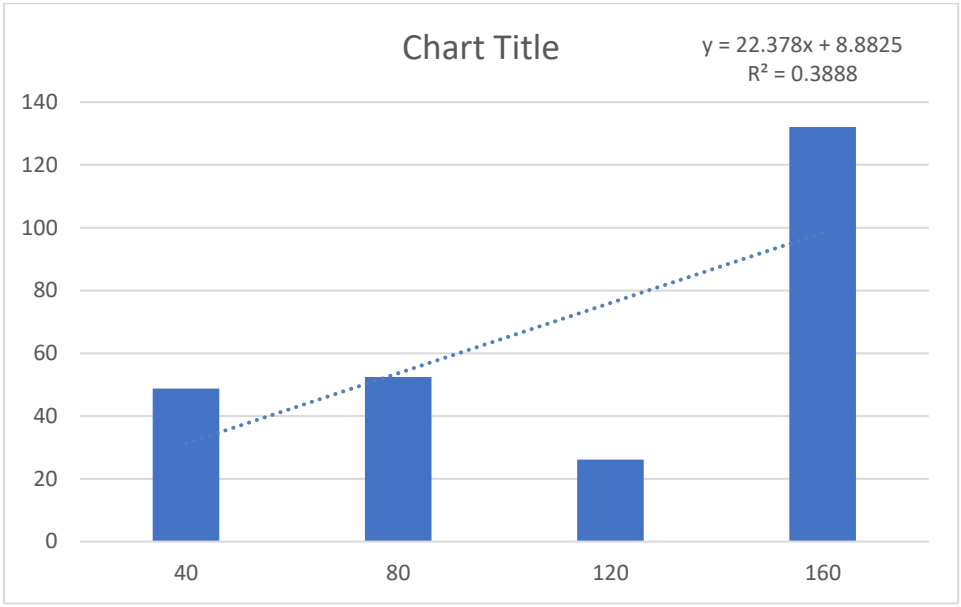
Cell cytotoxicity %



cell+C.P		
40	37.822	
80	51.575	
120	46.446	3.536415
160	50.507	



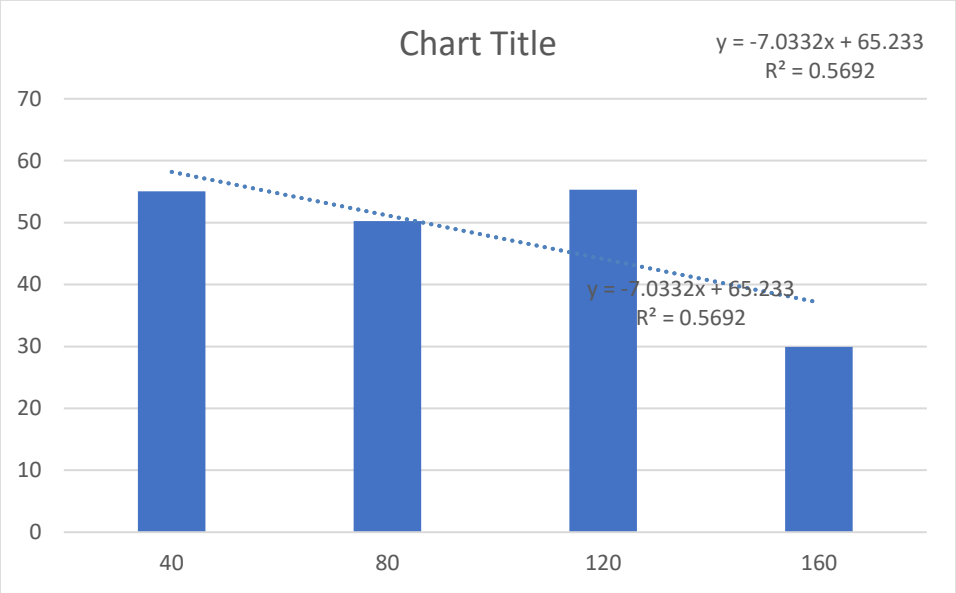
Cell+NP		
40	24.64	
80	13.451	9.263953
120	13.1979	
160	38.324	



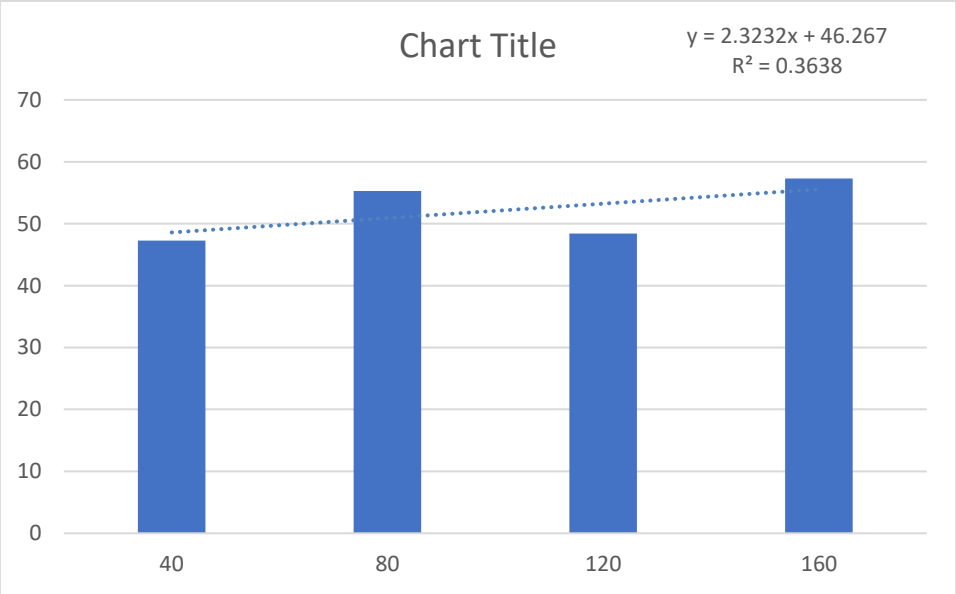
cell+curcumin		
40	72.493	
80	70.487	5.392011
120	72.206	
160	52.435	

Cell + inducer

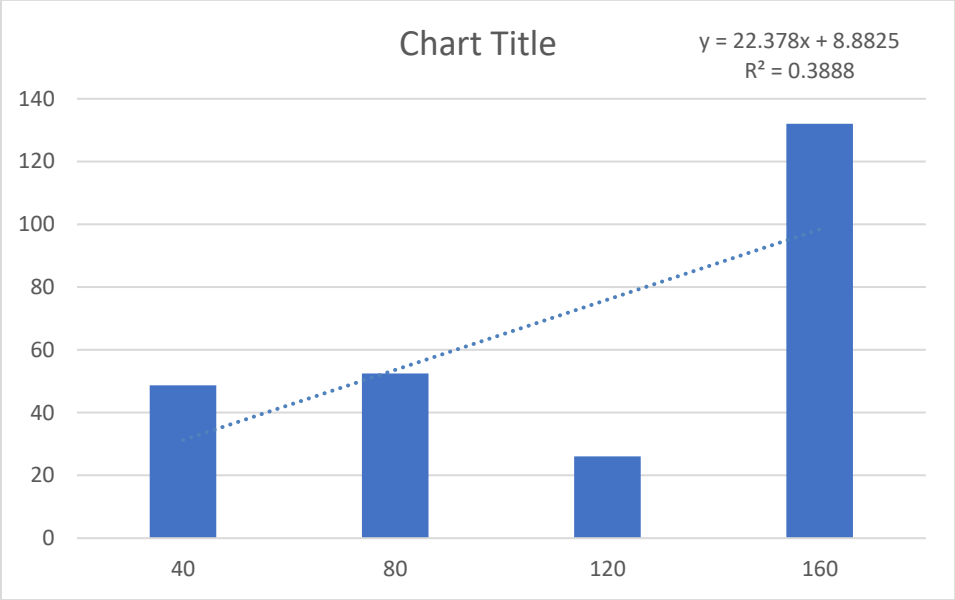
Cell + inducer 1



40	55.076		
80	50.253		ocadiac acid
120	55.329	2.16587	
160	29.94		

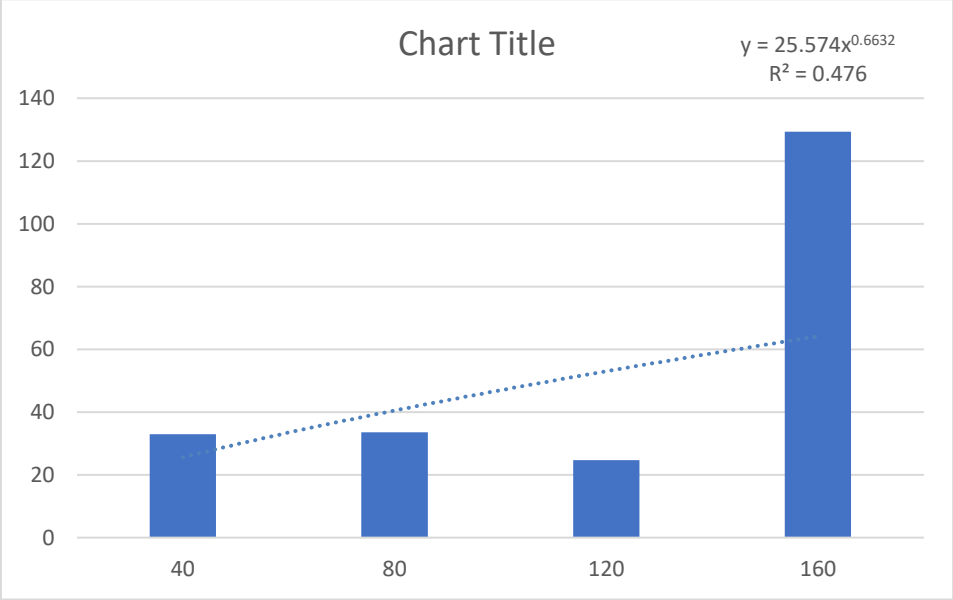


Cell+inducer2		
40	47.27	AlCl3
80	55.3	
120	48.424	1.606835
160	57.306	



Cell+inducer3

40	48.71	glyceraldehyde
80	52.435	
120	26.074	1.837407
160	132.09	



Cell+inducer4

40	32.951		
80	33.524	2.317575	Rotenon
120	24.64		
160	129.34		

Result and discussion

Fractions with highest concentration of curcumin were selected for HPLC analysis. 1mg/ml Standards of curcumin was prepared by dissolving in methanol. Working solutions (0.5–75.0 µg mL^{-1}) were made by diluting aliquots of the standard solution in methanol. Standards and fractions were syringe filtered through 0.45 µm. HPLC was used to evaluate curcumin, which was run on a C18 column with UV detection at 425 nm at a flow rate of 1.2 mL min^{-1}, the mobile phase was acetonitrile and water (50:50 v/v) acidified with 2

percent acetic acid Fractions having peaks with retention time similar to retention time of standard of curcumin were selected All the selected fractions were dried and weighed for encapsulation with synthesize calcium phosphate nanoparticles. Due to its ability to enhance bioavailability, reduce the dosage of harmful and expensive bioactive substances, and boost product performance, (Xia, atal,. 2018). Furthermore, amorphous calcium phosphate (ACP) nanoparticles possess a size in the nanometer range, and their high surface to volume ratio determines their high reactivity, solubility, biocompatibility, and bioactivity. As thus, ACP offers new opportunities in nanobiotechnology, particularly for improving the solubility, biocompatibility, and bioactivity of antioxidant plants including curcumin,(19). The co-precipitation method at 8pH values was successful in synthesizing amorphous calcium phosphate nanoparticles. IR spectroscopy, DLS, while SEM shows the encapsulation of curcumin and calcium phosphates nanoparticles, thus images of synthetic ACP have been shown in Figures 4a–4c that e and d Exhibit ACP nanoparticles in their dry form.

Samples	IC50 concentration(µg/ml)
Curcumin:	127.8305332
Calcium phosphate:	98.46698113
Encapsulated NP:	195.210122
Inducer for Alzheimer's disease	
Inducer1 (okadaic acid):	119.5099399
Inducer2 (AlCl3):	108.3870968
Inducer3 (Glyceraldehyde):	92.56163811
Inducers for Parkinson's disease	
Inducer 4 (Rotenone):	75.12713823

Conclusion

The synthesis of calcium phosphate nanoparticles was carried out utilizing a simple, quick and easy surfactant-free, and environmentally friendly appropriate procedure, in which an aqueous solution containing Ca(NO3)2 was employed as a calcium source and (NH4)2HPO4 as a phosphorus source. The temperature, time frame, pH, and (NH4)2HPO4 concentrations of the reaction have a major impact on the product's crystal phase. calcium phosphate nanoparticles improved pH-responsive drug release property and a high Curcumin drug loading capacity, consequently, the calcium phosphate nanoparticles that have been loaded with curcumin will exhibit enhanced radical scavenging abilities and a strong fight neurological diseases .

References

1. Abirami, M., Raja, M. J., Mekala, P., & Visha, P. (2018). Preparation and Characterisation of Nanocurcumin Suspension, *7*(1), 100–103. Retrieved from www.ijset.net
2. Alabdali, A., Kzar, M., Chinnappan, S., Mogana, R., Khalivulla, S. I., Rahman, H., & Abd Razik, B. M. (2021, December 1). Antioxidant activity of Curcumin. *Research Journal of Pharmacy and Technology*. Research Journal of Pharmacy and Technology. https://doi.org/10.52711/0974-360X.2021.01164
3. Amato, A., Terzo, S., & Mulè, F. (2019, December 1). Natural compounds as beneficial antioxidant agents in neurodegenerative disorders: A focus on Alzheimer's disease. *Antioxidants*. MDPI. https://doi.org/10.3390/antiox8120608
4. Ashok, A., Andrabi, S. S., Mansoor, S., Kuang, Y., Kwon, B. K., & Labhasetwar, V. (2022, February 1). Antioxidant Therapy in Oxidative Stress-Induced Neurodegenerative Diseases: Role of Nanoparticle-Based Drug Delivery Systems in Clinical Translation. *Antioxidants*. MDPI. https://doi.org/10.3390/antiox11020408
5. Askarizadeh, A., Barreto, G. E., Henney, N. C., Majeed, M., & Sahebkar, A. (2020, July 30). Neuroprotection by curcumin: A review on brain delivery strategies. *International Journal of Pharmaceutics*. Elsevier B.V. https://doi.org/10.1016/j.ijpharm.2020.119476
6. Barick, K. C., Tripathi, A., Dutta, B., Shelar, S. B., & Hassan, P. A. (2021).

Curcumin Encapsulated Casein Nanoparticles: Enhanced Bioavailability and Anticancer Efficacy. *Journal of Pharmaceutical Sciences*, *110*(5), 2114–2120. https://doi.org/10.1016/j.xphs.2020.12.011

7. Bhalani, D. V., Nutan, B., Kumar, A., & Singh Chandel, A. K. (2022, September 1). Bioavailability Enhancement Techniques for Poorly Aqueous Soluble Drugs andTherapeutics. *Biomedicines*.MDPI.https: //doi.org/10.3390/biomedicines10092055
8. Brain, I., & Delivery, T. (2021). Its Brain Targeted Delivery.
9. Buckner, C. A., Lafrenie, R. M., Dénommée, J. A., Caswell, J. M., & Want, D. A. (2018). Complementary and alternative medicine use in patients before and after a cancer diagnosis. *Current Oncology*, *25*(4), e275–e281. https://doi.org/10.3747/co.25.3884
10. Chen, M., Du, Z. Y., Zheng, X., Li, D. L., Zhou, R. P., & Zhang, K. (2018, April 1). Use of curcumin in diagnosis, prevention, and treatment of Alzheimer's disease. *Neural Regeneration Research*. Wolters Kluwer Medknow Publications. https://doi.org/10.4103/1673-5374.230303
11. Del Prado-Audelo, M. L., Caballero-Florán, I. H., Meza-Toledo, J. A., Mendoza-Muñoz, N., González-Torres, M., Florán, B. Leyva-Gómez, G. (2019). Formulations of curcumin nanoparticles for brain diseases. *Biomolecules*. MDPI AG. https://doi.org/10.3390/biom9020056
12. El Nebrisi, E. (2021, October 1). Neuroprotective activities of curcumin in parkinson's disease: A review of the literature. *International Journal of Molecular Sciences*. MDPI. https://doi.org/10.3390/ijms222011248
13. Klran, T. R., Otlu, O., & Karabulut, A. B. (2023, February 1). Oxidative stress and antioxidants in health and disease. *Journal of Laboratory Medicine*. Walter de Gruyter GmbH. https://doi.org/10.1515/labmed-2022-0108
14. Klran, T. R., Otlu, O., & Karabulut, A. B. (2023, February 1). Oxidative stress and antioxidants in health and disease. *Journal of Laboratory Medicine*. Walter de Gruyter GmbH. https://doi.org/10.1515/labmed-2022-0108
15. Lamptey, R. N. L., Chaulagain, B., Trivedi, R., Gothwal, A., Layek, B., & Singh, J. (2022, February 1). A Review of the Common Neurodegenerative Disorders: Current Therapeutic Approaches and the Potential Role of Nanotherapeutics. *International Journal of Molecular Sciences*. MDPI. https://doi.org/10.3390/ijms23031851
16. Pan, K., Zhong, Q., & Baek, S. J. (2013). Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. *Journal of Agricultural and Food Chemistry*, *61*(25), 6036–6043. https://doi.org/10.1021/jf400752a
17. Priyanka, R., Vasundhara, M., Rao, G. G. E., Thara, B. S., Radhika, B., & Marappa, N. (2017). Antioxidant activity of turmeric (Curcuma longa L.) cultivars. *Medicinal Plants*, *9*(3), 189–194. https://doi.org/10.5958/0975-6892.2017.00029.6
18. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., … Sharifi-Rad, J. (2020, July 2). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in Physiology*. Frontiers Media S.A. https://doi.org/10.3389/fphys.2020.00694
19. Sies, H. (2020). Oxidative stress: Concept and some practical aspects. *Antioxidants*, *9*(9), 1–6. https://doi.org/10.3390/antiox9090852
20. Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019, April 22). Oxidative stress: A key modulator in neurodegenerative

diseases. *Molecules*. MDPI AG. https://doi.org/10.3390/molecules24081583

21. Tanvir, E. M., Hossen, M. S., Hossain, M. F., Afroz, R., Gan, S. H., Khalil, M. I., & Karim, N. (2017). Antioxidant properties of popular turmeric (Curcuma longa) varieties from Bangladesh. *Journal of Food Quality*, *2017*. https://doi.org/10.1155/2017/8471785
22. Vecstaudza, J., & Locs, J. (2017). Novel preparation route of stable amorphous calcium phosphate nanoparticles with high specific surface area. *Journal of Alloys and Compounds*, *700*, 215–222. https://doi.org/10.1016/j.jallcom.2017.01.038
23. Xia Guo, Wenfeng Li, Heping Wang, Yan-Ying Fan, Huifang Wang, Xianghua Gao, Baolong Niu, Xuechen Gong, Preparation, characterization, release and antioxidant activity of curcumin-loaded amorphous calcium phosphate nanoparticles, journal of Non-Crystalline Solids, Volume 500, 2018, Pages 317-325, ISSN 0022-3093, https://doi.org/10.1016/j.jnoncrysol.2018.08.015.
24. Yesiloglu, Y., & Aydin, H. (2013). Evaluation of antioxidant activities of turmeric extracts from Turkey. *Asian Journal of Chemistry*, *25*(13), 7199–7204. https://doi.org/10.14233/ajchem.2013.14503
25. Zhai, K., Brockmüller, A., Kubatka, P., Shakibaei, M., & Büsselberg, D. (2020, November 1). Curcumin's beneficial effects on neuroblastoma: Mechanisms, challenges, and potential solutions. *Biomolecules*. MDPI AG. https://doi.org/10.3390/biom10111469